Robust Summaries for

Tertiary Butanol

CAS Number 75-65-0

USEPA HPV Challenge Program
Final Submission

November 24, 2004

Submitted by:

Propylene Carbonate / t-Butyl Alcohol HPV Committee

Members:

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Data Point	Method	Value
1) MELTING POINT	Not Stated	25°C
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
2) BOILING POINT	Not Stated	81°C
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
3) VAPOUR PRESSURE	Not Stated	31 mm Hg @25°C
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
4) PARTITION COEFFICIENT	Not stated	Log Pow: 0.37 Temp: Not stated
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
5) WATER SOLUBILITY	Not stated	soluble in water
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
	Not stated	1000 g/l @25°C
Reference: Riddick, J.A., Bunger, W.B., Sakano T.K. 1985. Techniques of Chemistry 4th ed., Volume II. Organic Solvents. New York, NY: John Wiley and Sons.		
6) PHOTODEGRADATION	Not required	does not absorb in region of 290-800 nm
Reference: US EPA test Guideline 835.2310		

8) TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS (FUGACITY)

TEST SUBSTANCE

Identity: t-butanol
Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Test (test type): Model estimation - EPIWIN
Method (Y/N): Y
Year (study performed): 2002

Remarks field for Test Conditions. Detail the model used (title, version and date) and the input parameters (chemical-specific, environmental conditions) as necessary.
Model = EPIWIN v. 3.10
Input parameters: water sol. = 1E+06 mg/L
vapor pressure = 40.7 mm Hg
Henry LC = 9.05E-0.06 atm-m3/mole
Log Kow = 0.35
boiling point = 82.4 deg C
melting point = 25.4 deg C

RESULTS

Estimated Distribution and Media Concentration (levels II/III):
Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	9.5	229	1000
Water	50.4	360	1000
Soil	40	360	1000
Sediment	0.0856	1440	0

Persistence Time: 287 hr

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use including the following if available:
- Soil Adsorption coefficient: 1.471 (PCKOCWIN v. 1.66)

CONCLUSIONS: t-Butanol will partition mostly to water and soil.

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Conclusion of submitter.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study') = 1

Remarks field for Data Reliability: Used EPA software based on published method.

REFERENCES (Free Text): EPISuite, USEPA software v3.10, downloaded from EPA Website 2002.

OTHER

Last changed (administrative field for updating): 10/10/02 by ToxWorks

9) BIODEGRADATION

9.1 OECD 301B

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.) Commercial t-butanol, 99.7% pure

METHOD

Method/guideline followed (include calculated as one of the possible methods): OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Test Type (test type/aerobic/anaerobic): Aerobic
GLP (Y/N): Yes
Year (study performed): 2003
Contact time (units): 28 days
Innoculum: Activated sludge, domestic

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations, whether there was bacterial inhibition, and detail differences from the guideline followed including the following as appropriate:

Deionized, purified, filtered water was used for this study. The microbial inoculum was activated sludge from the Columbia Wastewater Treatment Plant, Columbia, MO, which treats predominately domestic sewage. The sludge was prepared by filtering through glass wool; each reaction flask contained 1 mg/l of suspended solids. The activated sludge contained 2.6×10^6 colony forming units/ml of microorganisms, or 7.6×10^4 CFU/ml in the reaction flasks. To remove CO_2, the incoming air was passed through an Ascarite column, followed by a trap of 5N KOH.

2.4 L of the test medium was placed in each of 5 5L flasks, with 30 ml of activated sludge, and aerated and stirred for 24 hours prior to addition of test or reference compound. Reaction flasks were chosen at random for control 1, control 2, t-butanol 1, t-butanol 2, or sodium benzoate, reference compound. T-Butanol was added to create a solution of 20 mg/l carbon, by addition of 93 mg t-butanol to the each of the two replicates. Sodium benzoate solution was added to the reference flask to generate a solution of 20 mg/l carbon. Additional water was added to each of the flasks to give a total volume of 3 l.

The flasks were incubated in the dark at 22 C and stirred for 29 days with continual aeration by 50-100 ml/min CO_2-free air. Off-gases were passed through 3 100 ml 0.2N KOH traps; analysis for CO_2 was performed on Days 2, 5, 7, 9, 14, 19, 23, 28, and 29. After day 28, an aliquot was removed from each reaction flask and analyzed for total carbon and inorganic carbon. Dissolved organic carbon (DOC) was calculated as the difference between total carbon and inorganic carbon.

RESULTS

Degradation % after time: 2.6-5.1% after 29 days
Results: not readily biodegradable

Remarks field for Results (Describe additional information that may be needed to adequately assess data for reliability and use, e.g. lag time, observed inhibition, excessive biodegradation, excessive standard deviation, kinetics, time required for 10% degradation and total degradation at the end of the test.)

The evolution of CO_2 was 2.6% and 5.1% of the theoretical CO_2 collected in the traps after 29 days.

In the control solution, DOC was 1.62 mg C/l at study initiation and 2.12 mg C/l at termination. These values were subtracted from the DOC values for the test flasks. For t-butanol, the DOC was 20.2 and 19.9 mg C/l in the two replicates at initiation, and 9.29 and 7.39 mg C/l at termination. Thus, 54.0% and 62.9% for replicates 1 and 2, respectively, of the original DOC from t-butanol was removed during the biodegradation study.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: The author concludes that t- butanol is not readily biodegradable.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability: This study was conducted in a reliable laboratory according to the current test guideline and GLPs.

REFERENCES (Free Text): Belarde D. (2003). Determination of the Ready Biodegradability of Tert-Butyl Alcohol Using the CO_2 Evolution Method. OECD 301. ABC Study No. 48067, ABC Laboratories, Inc., Columbia, Missouri 65202, pp. 1-50.

OTHER

Last changed (administrative field for updating): 1/16/04 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

9.2 Comparison of Methods

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed (include calculated as one of the possible methods): Zahn-Wellens; MITI; Sturm; OECD Screen; Closed bottle
Test Type (test type/aerobic/anaerobic): Aerobic

GLP (Y/N): Not reported
Year (study performed): Not reported
Contact time (units): Varied by test
Inoculum: Varied by test

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations, whether there was bacterial inhibition, and detail differences from the guideline followed including the following as appropriate:

RESULTS

Degradation % after time
Results: Zahn-Wellens – 96% removed after 6 days
MITI - 0% removed after 14 days
Sturm – 32% removed, 0% CO2 evolved
OECD screen – 29% converted
Closed bottle – 0% BODT at 30 days

Remarks field for Results (Describe additional information that may be needed to adequately assess data for reliability and use, e.g. lag time, observed inhibition, excessive biodegradation, excessive standard deviation, kinetics, time required for 10% degradation and total degradation at the end of the test.)

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Degree of biodegradation very dependent on method used.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2, Key study

Remarks field for Data Reliability: Limited details, but important because several methods are compared.

REFERENCES (Free Text): Gerike, P. and Fischer, W.K., 1979. A correlation study of biodegradability determinations with various chemicals in various tests. Ecotoxicol. Environ. Safety 3: 159-173.

OTHER

Last changed (administrative field for updating): 3/12/02 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

ECOTOXICITY ELEMENTS

10) ACUTE TOXICITY TO FISH

10.1 Fathead Minnow

TEST SUBSTANCE

Identity : t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.) Commercial t-butanol, 99.7% pure

METHOD

Method/guideline followed (experimental/calculated): U.S. EPA OPPTS Guideline 850.1075; OECD Guideline 203
Type (test type): Flow-through
GLP (Y/N): Yes
Year (study performed):2003
Species/Strain/Supplier: Fathead Minnow, *Pimephales promelas* from in-house culture
Analytical monitoring: gas chromatography
Exposure period (unit): 96 hours
Statistical methods: None needed to evaluate the data

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations, and detail differences from the guideline followed including the following as appropriate:

- Test fish (Age/length/weight, loading, pretreatment):
  Mean Standard Length = 17 ± 2.1 mm (12 to 21)
  Mean Total Length = 22 ± 2.4 mm (19 to 27)
  Mean blotted wet weight = 0.082 ± 0.029 g (0.039 to 0.149)
  Dynamic loading = 0.011 g/L/day
- Test conditions, e.g. :
  · Details of test (static, semi-static, flow-through): Flow-through
  · Dilution water source: ABC labs, moderately hard prepared by blending naturally hard well water with well water that was demineralized by reverse osmosis.
  · Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity): total hardness of 130 to 160 mg $CaCO_3$/L, pH = 8.3-8.4
  · Stock and test solution and how they are prepared: An intermittent-flow proportional diluter system similar to that described by Mount and Brungs, with a

Hamilton Model 420 syringe injector was used for the intermittent introduction of an aqueous solution of t-butanol and control dilution water to exposure chambers. The diluter system provided five test substance treatment levels with a 50% dilution factor between treatments and delivered approximately 1 L to each test chamber with each cycle. Diluter stock solutions were prepared in deionized water at a target concentration of 390,500 mg a.i./L by mixing 391.7 g of test substance into 1 L of deionized water. Stock solution usage was monitored on a daily basis. The diluter system was volumetrically calibrated before test initiation. Proper operation of the diluter and all mechanical systems was verified at least twice each day. The diluter system was labeled with study number and treatments.

· Concentrations dosing rate, flow-through rate, in what medium: Nominal concentrations = 1000, 500, 250, 125 and 62.5 mg /l; flow through rate = 80 l/day in chambers holding 15 l.

· Vehicle/solvent and concentrations: No vehicle or solvent used, t-butanol dissolved in test water to make stock solution

· Stability of the test chemical solutions: Not stated, concentrations determined day 0 and 4.

· Exposure vessel type (e.g., size, headspace, sealed, aeration, lighting, # per treatment): Glass tank - 23 x 31 x 32 cm, filled with 15 l (~2/3rds full); open; fluorescent lighting 16 hours/day providing 732 lux at the surface, 2 replicates/treatment concentration.

· Number of replicates, fish per replicate: 2 replicate, 10 fish each; 20 fish/concentration

· Water chemistry in test (D.O., pH) in the control and one concentration where effects were observed: D.O.= 7.8-9.1 mg/l; pH = 8.3-8.4

- Test temperature range - Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): 22.9 to 23.9°C

RESULTS

Nominal concentrations (as mg/L):		0,	62.5,	125,	250,	500,	1000
Measured concentrations (as mg/L):	At initiation:	<42.2,	80.4,	147,	271,	525,	998
	At Day 4:	<42.2,	71.5,	122,	238,	465,	924
	Average of Day 0 and 4:	<42.2,	76.0,	135,	255,	495,	961

Unit (results expressed in what unit): mg/l

Element value (e.g. LC50, LClo, LL50, or LL0 at 48, 72 and 96 hours, etc., based on measured or nominal concentrations): LC_{50} >961 mg/l at 96 hours, based on average measured concentration

Statistical results, as appropriate

Remarks field for Results. Discuss if element effect concentration is greater than materials solubility. Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available:

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Submitter concludes that t-butanol has limited toxicity to fathead minnow.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1

Remarks field for Data Reliability: This study was conducted in a reliable laboratory according to the current test guideline and GLPs.

REFERENCES (Free Text): Hughes, CD. (2003). Acute Toxicity of Tert-Butyl Alcohol to the Fathead Minnow, *Pimephales promelas*, Determined Under Flow-Through Test Conditions; ABC Study No. 48066, ABC Laboratories, Inc., Columbia, Missouri 65202, pp. 1-24.

OTHER

Last changed (administrative field for updating): 1/16/04 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

10.2 Goldfish

TEST SUBSTANCE

Identity : t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed (experimental/calculated): Not stated
Type (test type): Static
GLP (Y/N): No data
Year (study performed): Not stated; published 1979
Species/Strain/Supplier: *Carassius auratus* (goldfish), strain and supplier not stated
Analytical monitoring: Total carbon or gas chromatography
Exposure period (unit): 24 hours
Statistical methods

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations, and detail differences from the guideline followed including the following as appropriate:

- Test fish (Age/length/weight, loading, pretreatment): Length = 6.2±0.7 cm;

Weight = 3.3 ±1.0 g

- Test conditions, e.g. :
  - · Details of test (static, semi-static, flow-through): Static
  - · Dilution water source: Not stated
  - · Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity): Not stated
  - · Stock and test solution and how they are prepared: Not stated
  - · Concentrations dosing rate, flow-through rate, in what medium: Not stated
  - · Vehicle/solvent and concentrations: Not stated
  - · Stability of the test chemical solutions: Not stated
  - · Exposure vessel type (e.g., size, headspace, sealed, aeration, lighting, # per treatment): Glass tank - 42 x 28 x 28 cm, open, not aerated due to volatility of TBA
  - · Number of replicates, fish per replicate: 1 replicate, 6 fish
  - · Water chemistry in test (D.O., pH) in the control and one concentration where effects were observed: Not stated

- Test temperature range - Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): 20±1° C

RESULTS

Nominal concentrations (as mg/L): Not stated
Measured concentrations (as mg/L): Not stated
Unit (results expressed in what unit): mg/l
Element value (e.g. LC50, LClo, LL50, or LL0 at 48, 72 and 96 hours, etc., based on measured or nominal concentrations): >5000 mg/l at 24 hours
Statistical results, as appropriate

Remarks field for Results. Discuss if element effect concentration is greater than materials solubility. Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available:

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Submitter concludes that insufficient details are available to adequately characterize acute toxicity of t-butanol to fish.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 3

Remarks field for Data Reliability: Not guideline, poor documentation

REFERENCES (Free Text): Bridie, A.L., Wolff, C.J.M., and Winter, M., 1979. The acute toxicity of some petrochemicals to goldfish. Water Research 13: 623-626.

OTHER

Last changed (administrative field for updating): 3/12/02 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

11) TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks. Commercial t-butanol, 99.7% pure

METHOD

Method/guideline followed (experimental/calculated): U.S. EPA OPPTS Guideline 850.5400; OECD Guideline 201
Test type: 96 hour based on growth rate
GLP (Y/N): Yes
Year (study performed): 2003
Species/Strain: unicellular green alga, *Selenastrum capricornutum*
Test details (static, semi-static, dosing rate, flow-through rate, etc.): Static
Statistical methods: All statistical analyses were performed using SAS software. The NOEC's, based on cell density, area under the growth curve, and growth rate, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test.

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Test organisms: unicellular green alga, *Selenastrum capricornutum*, obtained from the Department of Botany, Culture Collection of Algae, University of Texas at Austin, on January 22, 2003. The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new *Selenastrum* cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing. The algal culture used for this test was three days old at test initiation.

- Test conditions
  - · Stock solutions preparation (vehicle, solvent, concentrations) and stability: t-butanol prepared at 1 mg/ml and confirmed by GC analysis

· Test temperature range: 23.8 to 24.5°C.
· Exposure vessel type (e.g., size, headspace, sealed, aeration, # per treatment): 550 ml Erlenmeyer flask, 3 replicates per dose level
· Dilution water source: The test medium was freshwater algal nutrient medium (FWAM) containing silicon and prepared in ABC reagent water. After preparation, the medium was pH-adjusted to 7.5 ± 0.1 using 0.1 N HCl and 0.1 N NaOH and filtered through a 0.45-µm Millipore® filter.
· Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity, Ca/Mg ratio, Na/K ratio): Below limit of quantitation for 36 organic compounds and 12 metals
· Lighting (quality, intensity and periodicity): Continuous lighting was provided at an average light intensity of 4,271 ± 117 lux.
· Temprature: 24.2-24.5 C
· Water chemistry in test (D.O., pH) in the control and at least one concentration where effects were observed: pH measured daily in all treatment groups; range 7.4 to 9.2

- Element (unit) basis (i.e. immobilization): Growth, based on Area under the curve, and growth rate
- Test design (number of replicates, individuals per replicate, concentrations): 3 replicates/concentration; 5 concentrations tested; 1 x 10^4 algae added/replicate
- Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): Microscope evaluation using hemacytometer
- Exposure period: 96 hours
- Analytical monitoring: GC analysis of t-butanol concentration at 0, 72, and 96 hours

RESULTS

Nominal concentrations in mg/L: 0, 62.5, 125, 250, 500, 1000 mg/l
Measured concentrations in mg/L:
at initiation: <MQL , 67.5, 124, 254, 494, 976 mg/l
at 72 hours: <MQL, 42.3, 90.4, 182, 369, 770 mg/l
at 96 hours: <MQL, 49.1, 102, 194, 332, 695 mg/l
MQL = 42.2 mg/l
EC50, EL50, LC0, LL0, at 24, 48 hours: EC_{50}= >976 mg/l
Statistical results, as appropriate

Remarks field for Results. Discuss if element effect concentration is not less than materials solubility. Describe additional information that may be needed to adequately assess data for reliability and use including the following as appropriate:
After 96 hours of exposure, mean cell density in the control was 195 × 10^4 cells/mL, or 195 times the initial inoculum. The coefficient of variation was 8.0% for the control. The mean cell density in the t-butanol treatments ranged from a low of 204 × 10^4 cells/mL at a concentration of 67.5 mg total product/L to a high of 225 × 10^4 cells/mL at a concentration of 976 mg total product/L. Percent difference in algal growth ranged from +4.6% at a concentration of 67.5 mg total product/L to +15% at a concentration of 976 mg total product/L. Growth curves for the

control and t-butanol treatments showed no differences. After 72 and 96 hours of exposure, there was no statistically significant reduction in cell density, area under the growth curve, or growth rate.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: The submitter concludes that t-butanol is not toxic to algae.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability: This study was conducted in a reliable laboratory according to the current test guideline and GLPs.

REFERENCES (Free Text): Hughes, C. (2003). Toxicity of Tert-Butyl Alcohol to the Unicellular Green Alga, *Selenastrum capricornutum*. ABC Study No. 48065, ABC Laboratories, Inc., Columbia, Missouri 65202, pp. 1-35.

OTHER

Last changed (administrative field for updating): 1/16/04 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

12) ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.

METHOD

Method/guideline followed (experimental/calculated): German Institute of Standardization, 1982 DIN 38412, Part II.
Test type: Acute daphnia
GLP (Y/N): Not stated
Year (study performed): Not stated
Analytical procedures: Not stated

Species/Strain: *Daphnia magna* in-house cultures
Test details (static, semi-static, dosing rate, flow-through rate, etc.): Static
Statistical methods: Not stated

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Test organisms: No details provided.

- Test conditions
  - · Stock solutions preparation (vehicle, solvent, concentrations) and stability: No solvent; stock solution in water, diluted with test water.
  - · Test temperature range: 20±1°C
  - · Exposure vessel type (e.g., size, headspace, sealed, aeration, # per treatment): 50 ml beaker, open, 2 used per concentration
  - · Dilution water source: Not stated
  - · Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity, Ca/Mg ratio, Na/K ratio): Acid capacity ($K_{s4.3}$) = 0.8mmol per l; hardness 2.4 mmol per l; Ca:Mg = 4:1; Na:K = 10:1; initial pH = 8.0±0.2
  - · Lighting (quality, intensity and periodicity): Not stated
  - · Water chemistry in test (D.O., pH) in the control and at least one concentration where effects were observed: D.O. > 2 mg/l.
- Element (unit) basis (i.e. immobilization): Immobilization
- Test design (number of replicates, individuals per replicate, concentrations): 2 replicates/concentration; 10 individuals/replicate
- Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): Not stated
- Exposure period: 48 hours
- Analytical monitoring: Not stated

RESULTS

Nominal concentrations in mg/L: Not stated
Measured concentrations in mg/L: Not stated
Unit [results expressed in what unit]: mg/l
EC50, EL50, LC0, LL0, at 24, 48 hours: EC_{50}= 5504 (4607-6577) mg/l
Statistical results, as appropriate

Remarks field for Results. Discuss if element effect concentration is not less than materials solubility. Describe additional information that may be needed to adequately assess data for reliability and use including the following as appropriate:

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: The submitter concludes that t-butanol is not toxic to invertebrates.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2, Key study

Remarks field for Data Reliability: Appears to meet most of requirements of EPA OPPTS 850.1010, but some details not reported

REFERENCES (Free Text): Kuhn, R., Pattard, M., Pernak, K.-D., and Winter, A., 1989. Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna*. Wat. Res. 23: 495-499.

OTHER

Last changed (administrative field for updating): 11/21/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

HEALTH ELEMENTS

13. Acute Toxicity

13.1 Acute Oral Toxicity

Overview

Three acute oral toxicity studies on t-butyl alcohol have been performed. The results of all three studies should be considered together because they all provide solid evidence of the clinical and pathologic endpoints observed following a single oral dose of t-butanol. The following table tabulates the results:

Species	LD_{50} (mg/kg)	Results	Reference
Rat	2733	Ataxia, prostration, Bradypnea	IRDC, 1981
Rat	3500	Ataxia	Schaffarzick and Brown, 1952
Rabbit	3558	Dyspnea, Bradycardia, corneal reflexes	Munch, 1972

13.1.1 Acute oral toxicity in rats

IDENTITY: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD:

Method/guideline followed: EPA
Type: Acute oral
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain: Rat, Sprague Dawley; Charles River, Portage MI
Sex: Both
No. of animals per dose: 5 per sex per dose
Vehicle: Not reported
Route of administration: Oral via stomach tube

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age: Not reported
- Weight: 200 to 280 g
- Doses: 1500, 1950, 2535, 3296, and 4285 mg/kg
- Doses per time period: Presumed 1
- Volume administered or concentration: Not reported
- Post dose observation period: 14 days

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LD_{50} (combined) = 2733 (2249-3320) mg/kg
Males: 3046 (2581-3596); Females 2298 (1767-2987)

Number of deaths at each dose level:

(mg/kg)	1500	1950	2535	3296	4285
Males:	0/5	0/5	3/5	1/5	5/5
Females:	0/5	3/5	2/5	4/5	5/5
Total	0/10	3/10	5/10	5/10	10/10

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available:

Ataxia, prostration, piloerection, bradypnea and hypoactivity were observed in the animals of all groups. Generally, surviving animals appeared normal by day 6.

Incidences were:	1500		1950		2535		3296		4285	
	M	F	M	F	M	F	M	F	M	F
Ataxia	5	4	5	3	3	5	4	4	1	2
Piloerection	5	5	5	5	5	5	5	5	5	4
Prostration	3	0	3	3	2	3	3	4	5	5
Bradypnea	3	3	2	2	1	2	3	4	5	5
Hypoactivity	2	3	5	2	2	3	4	3	0	0

Body weights remained unchanged during the study.

No compound related macroscopic lesions were noted.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1 – Key Study

Remarks field for Data Reliability

REFERENCE: International Research and Development Corporation. 1981. Acute Oral Toxicity (LD_{50}) in Rats (EPA 8/78). Final Report 419-019, August 3, 1981

OTHER

Last changed: 10/08/02 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

13.1.2 Acute oral toxicity in rats

IDENTITY: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD:

Method/guideline followed: No details provided
Type: Acute oral
GLP (Y/N): No
Year (study performed): Not stated
Species/Strain: Rat, strain not stated

Sex: Not reported
No. of animals per dose: Not reported
Vehicle: Not reported
Route of administration: Oral, presumed gavage

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age: Not reported
- Doses: Not reported
- Doses per time period: Not reported
- Volume administered or concentration: Not reported
- Post dose observation period: Not reported. At various times following dosing, the animals were tested for anticonvulsant activity.

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LD_{50} = 3500 mg/kg
ED_{50} (anticonvulsant activity) = 59 mg/kg
ED_{50} (ataxia) = 530 mg/kg.

Number of deaths at each dose level: Not reported

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available: No details reported

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2

Remarks field for Data Reliability: Lacking method details, not guideline study, but appears satisfactory. Not chosen as key study because better study available.

REFERENCE: Schaffarzick, R.W. and Brown, B.J. (1952) The Anticonvulsant Activity and Toxicity of Methyl-parafynol and Some other Alcohols. Science 116 663 – 665.

OTHER

Last changed: 11/21/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

13.1.3 Acute oral toxicity in rabbits

IDENTITY: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD:

Method/guideline followed: Limited details provided
Type: Acute oral
GLP (Y/N): No
Year (study performed): Not stated, published 1972
Species/Strain: Rabbit, strain not stated
Sex: Not reported
No. of animals per dose: Not reported
Vehicle: Dose of TBA followed by 5 ml saline
Route of administration: Oral via stomach tube

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age: Not reported
- Weight: 1.5 to 2.5 kg
- Doses: Not reported
- Doses per time period: Not reported
- Volume administered or concentration: Administered neat; followed by 5 ml saline.
- Post dose observation period: Not reported. LD50 reported as deaths within 24 hours of dose.

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LD_{50} = 3558 mg/kg;
Reported by author as : LD_{50} = 48 mMoles/kg

Narcotic Dose (general stupor and loss of voluntary movements in 50% of the animals):
19 mMoles/kg (1408mg/kg)

Larger doses resulted in the observation of loss of corneal reflexes, nystagmus, dyspnea and bradycardia.

Number of deaths at each dose level: Not reported

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available: No details reported.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2

Remarks field for Data Reliability: Limited by lack of details, not guideline study. Not chosen as key study because better study available.

REFERENCE: Munch, J.C. (1972) Aliphatic Alcohols and Alkyl Esters: Narcotic and Lethal Potencies to Tadpoles and to Rabbits. Industrial Medicine 41: 31- 33.

OTHER

Last changed: 11/21/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

13.2 Acute Dermal Toxicity in Rabbits

IDENTITY: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD:

Method/guideline followed: EPA
Type: Acute dermal
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain: Rabbit, New Zealand White
Sex: Both
No. of animals per dose: 5 per sex per dose
Vehicle: None
Route of administration: Dermal, abraded skin

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age: Not reported
- Weight: 2.5 to 3.2 kg
- Doses: 2000 mg/kg
- Doses per time period: 1

- Volume administered or concentration: Administered neat to abraded skin
- Post dose observation period: 14 days

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LD_{50} = > 2000 mg/kg

Number of deaths at each dose level: No deaths

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available:

All animals exhibited erythema and fissuring of the skin, ranging from “very slight” to “moderate” and desquamation ranging from “very slight” to “slight”.

Two males and one female reportedly exhibited significant weight loss during the study period (body weights not provided in report).

Gross pathological examination found thickening and encrustation at the treated site. These changes were considered incidental and not related to treatment. Hemorrhage was also observed at the treatment site in one male and one female. No other observations were noted.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability

REFERENCE: International Research and Development Corporation, 1981. Acute Dermal Toxicity (LD50) Study in Rabbits (TSCA 7/79) (EPA 8/78) (OSHA). Final Report 419-020. July 28, 1981.

OTHER

Last changed: 3/11/02 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

13.3 Acute Inhalation Toxicity in Rats

IDENTITY: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD:

Method/guideline followed: EPA; exception: temperature and humidity not measured in chambers
Type: Acute inhalation
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain: Rat, Charles River CD (Sprague-Dawley derived) Portage, MI
Sex: Both
No. of animals per dose: 5 per sex per dose
Vehicle: Air
Route of administration: Inhalation, vapor

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age: Males: 52-56 days; females: 58-62 days
- Weight: Males: 257-287 g; females: 172-212 g
- Doses: 9700 and 14,100 ppm analyzed vapor concentration
- Doses per time period: 4 hours, whole body
- Post dose observation period: 14 days

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LC_{50} = > 14,100 ppm

Number of deaths at each dose level: No deaths at 9700 ppm, 3 of 10 deaths at 14,100 ppm

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available:

Ataxia and prostration were observed during the exposure period at both exposure levels. Animals at the higher dose level also exhibited dyspsnea. During the post exposure period, ataxia and dyspnea were observed in all animals of the lowest dose.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability

REFERENCE: International Research and Development Corporation, 1981. LC50 Acute Inhalation Toxicity Evaluation in Rats. Final Report 419-020. August 19, 1981.

OTHER

Last changed: 11/21/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

GENETIC TOXICITY ELEMENTS

14 GENETIC TOXICITY IN VIVO (CHROMOSOMAL ABERRATIONS)

14.1 Micronucleus Assay in Mice

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: NTP
Type (test type): Micronucleus
GLP (Y/N): No data
Year (study performed): 1996
Species: Mice
Strain: B6C3F1
Sex: Male
Route of administration: Intraperitoneal injection 3 days
Doses/concentration levels: 0, 312.5, 625, 1250 mg/kg/day
Exposure period: 3 daily injections
Statistical methods: Fisher's Exact and Trend test

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age at study initiation: Not stated
- No. of animals per dose: 4
- Vehicle: Not stated
- Duration of test: 4 days: 3 days of exposure, analysis 24 hours later
- Frequency of treatment: Daily
- Sampling times and number of samples: 1 (24 hours)

- Control groups and treatment: Negative control and positive control (cyclophosphamide, CPA at 15 mg/kg)
- Clinical observations performed (clinical pathology, functional observations, etc.): Not stated
- Organs examined at necropsy (macroscopic and microscopic): Not stated
- Criteria for evaluating results (for example, cell types examined, number of cells counted in a mouse micronucleus test): 2000 cells counted, evaluation based on # MN/1000 polychromatic erythrocytes (PCE).
- Criteria for selection of M.T.D.: Not stated

RESULTS

Effect on mitotic index or PCE/NCE ratio by dose level by sex: MN/1000 PCE

Negative control	2.3±1.0
Positive control	14.2±1.6
t-butanol at 312.5 mg/kg/day	1.5±0.5
t-butanol at 625 mg/kg/day	1.4±0.1
t-butanol at 1250 mg/kg/day	1.7±0.3

Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal)
NOAEL(NOEL) (C)/LOAEL(LOEL) (C): Negative
Statistical results, as appropriate: Positive control statistically significant; all TBA groups not significant

Remarks field for Results Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available:

- Mortality at each dose level by sex: None
- Mutant/aberration/mPCE/polyploidy frequency, as appropriate:
- Description, severity, time of onset and duration of clinical signs at each dose level and sex: Not stated
- Body weight changes by dose and sex: Not stated
- Food/water consumption changes by dose and sex: Not stated

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Submitter concludes that t- butanol does not induce micronuclei.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2, Key study

Remarks field for Data Reliability: Results are from NTP database; they are not published or from official report.

REFERENCES (Free Text): In-Vivo Cytogenesis Testing: Micronucleus Induction Results, NTP, From NIEHS Central Data Management, unpublished results, 1996.

OTHER

Last changed (administrative field for updating): 11/29/01
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

15) GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

15.1 Ames Assay

15.1.1 Ames Assay – EG&G Mason Study

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: Ames
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Reverse mutation assay
System of testing [bacterial, non bacterial]: Bacterial
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain or cell type and or cell line, bacterial or non-bacterial: *Salmonella typhimurium*, strains TA98, TA100, TA1535, TA1537, and TA1538
Metabolic activation: S-9 from male rat liver induced by Aroclor 1254.
Concentrations tested: 100, 500, 2500, 5000, 10,000 µg/plate
Statistical Methods: Doubling over background

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations. Detail differences from the guideline followed including the following as appropriate:

- Test Design: Ames
· Number of replicates: 3
· Frequency of Dosing: Preincubation at 37°C for 20 minutes before plating for 48 hours
· Positive and negative control groups and treatment: Negative control and positive control for each strain: with activation: 2-aminoanthracene; without activation:

TA98, TA 1538 – 2-nitroflurene; TA100, TA 1535 – 1,3-Propane Sultone; TA 1537 – 9-Amionacridine

- Solvent: None
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Doubling of revertants over negative control value.

RESULTS

Cytotoxic concentration

- With metabolic activation: >10,000 µg/plate
- Without metabolic activation: >10,000 µg/plate

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)

- With metabolic activation: Negative
- Without metabolic activation: Negative

Statistical results, as appropriate

Remarks field for Results. Note test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they effect the selection of test concentrations or interpretation of the results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Author concluded that t-butanol did not induce mutations in Ames assay.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability: High Quality; best documented with full study details.

REFERENCES: EG&G Mason Research Institute, 1981. Salmonella/Mammalian-Microsome Preincubation Mutagenicity Assay. T-Butyl Alcohol. Final Report 052-398-635-2, EG&G Mason, May 8, 1981.

OTHER

Last changed (administrative field for updating): 3/11/02 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

15.1.2 Ames Assay – NTP Study

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: NTP Procedure
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Reverse mutation assay
System of testing [bacterial, non bacterial]: Bacterial
GLP (Y/N): Not stated
Year (study performed): Not stated
Species/Strain or cell type and or cell line, bacterial or non-bacterial: *Salmonella typhimurium*, strains TA98, TA100, TA1535, and TA1537
Metabolic activation: S-9 from male rat and male hamster liver induced by Aroclor 1254.
Concentrations tested: 100, 333, 1000, 3333, 10,000 µg/plate
Statistical Methods: Not stated

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations. Detail differences from the guideline followed including the following as appropriate:

- Test Design: Ames
· Number of replicates: 3
· Frequency of Dosing: Preincubation at 37°C for 20 minutes before plating for 48 hours
· Positive and negative control groups and treatment: Negative control and positive control for each strain: positive controls not explicitly stated
- Solvent: None
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Dose-related increase in revertants in any strain

RESULTS

Cytotoxic concentration
- With metabolic activation: >10,000 µg/plate
- Without metabolic activation: >10,000 µg/plate

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)
- With metabolic activation: Negative
- Without metabolic activation: Negative

Statistical results, as appropriate

Remarks field for Results. Note test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they effect the selection of test concentrations or interpretation of the results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Submitter concludes the study is negative

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study;

Remarks field for Data Reliability: High Quality; equally good quality and same results as previous study.

REFERENCES: Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. 1987. Salmonella mutagenicity tests. III. Results from testing of 225 chemicals. Environ. Mutagen. 9(Suppl. 9): 1-109.

OTHER

Last changed (administrative field for updating): 3/11/02 ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

15.1.3 Ames Assay – TA102

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.) 99.7% pure

METHOD

Method/guideline followed: OECD 471

Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Reverse mutation assay using Salmonella typhimurium strain TA102

System of testing [bacterial, non bacterial]: Bacterial

GLP (Y/N): yes

Year (study performed): 2003

Species/Strain or cell type and or cell line, bacterial or non-bacterial: *Salmonella typhimurium*, strains TA102

Metabolic activation: S-9 from male rat liver induced by a combination of phenobarbital and β-naphthoflavone.

Concentrations tested: 100, 200, 500, 1000, 2500, 5,000 µg/plate

Statistical Methods: Not stated

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations. Detail differences from the guideline followed including the following as appropriate:

- Test Design: Ames
· Number of replicates: 3 per dose level, solvent and activation system, study repeated
· Frequency of Dosing: Added to top agar
· Positive and negative control groups and treatment: Negative (solvent) control for each experiment; mitomycin C used as positive control without S9, 2-aminoanthracene used with S9 as positive control.
- Solvent: Water or DMSO (t-butanol tested using water as solvent; tested separately using DMSO as solvent.)
- Description of follow up repeat study: Same as first
- Criteria for evaluating results (e.g. cell evaluated per dose group): Statistically significant dose-related increase in revertants or doubling of revertant rate of solvent controls

RESULTS

Cytotoxic concentration

- With metabolic activation: >5,000 µg/plate
- Without metabolic activation: >5,000 µg/plate

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)

- With metabolic activation: Negative using water or DMSO
- Without metabolic activation: Negative using water of DMSO

Statistical results, as appropriate: No statistically significant increases

Remarks field for Results. Note test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they effect the selection of test concentrations or interpretation of the results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

Williams-Hill et al. (2000) reported a near doubling of mutation frequency when t-butanol was tested in strain TA102 using water as a solvent and adding rat S9. There was a dose-related increase in mutant frequency between 800 and 2000 μg/plate, which decreased at 3000 and 4000 μg/plate. There is no indication of assessment of cytotoxicity, replicate analysis, statistical analysis, or GLP. Williams-Hill, D., Spears, C.P., Prakash, S., Olah, G.A., Shamma, T., Moin, T., Kim, L.Y., Hill, C.K., (2000). Mutagenicity studies of methyl-*tert*-butylether using Ames tester strain TA102. Mutation Res. 446: 15-21.

Testing of t-butanol in strain TA102 by Huntingdon Life Sciences using DMSO as a solvent did not find increased mutations with or without metabolic activation using rat liver S9. t-Butanol was tested in duplicate experiments using 5 to 5000 μg/plate. May, K., 2000. Tertiary butyl acetate bacterial mutation assay. Report to Lyondell Chemicals from Huntingdon Life Sciences, Report 039/002499, Huntingdon, Cambridgeshire, England, pp. 1-32.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Submitter concludes that t-butanol does not induce mutations in *Salmonella typhimurium* strain TA102.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study;

Remarks field for Data Reliability: High Quality; GLP guideline study, with support from another GLP guideline study.

REFERENCES: Callander, R.D. (2003). Tert-butanol and MTBE: Bacterial mutation assay in *S. typhimurium*. Report to Lyondell Chemicals from Central Toxicology Laboratory, Report CTL/YV6279, Alderly Park, Cheshire, UK. Pp. 1-25.

OTHER

Last changed (administrative field for updating): 4/12/04 ToxWorks
Order number for sorting (administrative field)

15.2 Mouse Lymphoma Assay

15.2.1 Mouse Lymphoma Assay – EG&G Mason Study

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: Mouse Lymphoma
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Forward mutation assay
System of testing [bacterial, non bacterial]: Mammalian cells
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain or cell type and or cell line, bacterial or non-bacterial: L5178Y mouse lymphoma cells
Metabolic activation: S-9 from male rat liver induced by Aroclor 1254.

- Species and cell type: Mouse, L5178Y TK+/- lymphoma cells Clone 3.7.2C
- Quantity: 600,000 cells/ ml
- Induced or not induced: Both
- Concentrations tested: 1.7 to 32 µl/ml
- Statistical Methods: Doubling over controls

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations. Detail differences from the guideline followed including the following as appropriate:

- Test Design
· Number of replicates: 2; also 2 studies
· Frequency of Dosing: Once
· Positive and negative control groups and treatment: Negative control and positive control: 0.5 and 1.0 µl/ml ethylmethanesulonate without activation and 5 and 7.5 µl/ml 7,12-dimethylbenzanthacene with activation.
- Solvent: Ethanol
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Doubling of mutant frequency over control

RESULTS

Cytotoxic concentration:
- With metabolic activation: 100 µl/ml
- Without metabolic activation: 100 µl/ml

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)

- With metabolic activation: - Negative
- Without metabolic activation: Negative
Statistical results, as appropriate

Remarks field for Results. Note test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they effect the selection of test concentrations or interpretation of the results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Author concludes that t-butanol does not induce mutations in mouse lymphoma cells.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability

REFERENCES (Free Text): EG&G Mason Research Institute, 1981. Evaluation of test articles t-Butyl Alcohol – 99.9% (MRI #635) & t-Butyl Alcohol – Arconol (MRI # 636) for Mutagenic Potential Employing the L5178Y TK+/- Mutagenesis Assay. EG&G Mason Report 052-398-635-7 and 052-399-636-7, August 6, 1981.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

15.2.2 Mouse Lymphoma Assay – NTP Study

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: NTP Methods
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Forward mutation assay
System of testing [bacterial, non bacterial]: Mammalian cells
GLP (Y/N): Not stated
Year (study performed): Not stated
Species/Strain or cell type and or cell line, bacterial or non-bacterial: L5178Y mouse lymphoma cells
Metabolic activation: S-9 from male rat liver induced by Aroclor 1254.

- Species and cell type: Mouse, L5178Y TK+/- lymphoma cells Clone 3.7.2C
- Quantity: 600,000 cells/ ml
- Induced or not induced: Both
Concentrations tested: 1.7 to 32 µl/ml
Statistical Methods: Pairwise comparison and trend test

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations. Detail differences from the guideline followed including the following as appropriate:

- Test Design
· Number of replicates: 2; study conducted twice
· Frequency of Dosing: Once
· Positive and negative control groups and treatment: Negative control and positive control: 15 µg/ml methylmethanesulonate without activation and 2.5 µl/ml MCA
- Solvent: Ethanol
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Significant increase in 1 of top 3 doses and significant trend

RESULTS

Cytotoxic concentration:
- With metabolic activation: >5000 µg/ml
- Without metabolic activation: >5000 µg/ml

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)

- With metabolic activation: - Negative
- Without metabolic activation: - Negative
Statistical results, as appropriate: Positive control positive, no test groups positive

Remarks field for Results. Note test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they effect the selection of test concentrations or interpretation of the results. Describe

additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Author concluded that t-butanol does not induce mutations in mouse lymphoma cells.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability

REFERENCES (Free Text): McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., and Caspary, W.J., 1988. Responses of the L5178 tk+/tk- mouse lymphoma cell forward mutation assay to coded chemicals. II. 18 coded chemicals. Environ, Molec. Mutagen. 11: 91-118.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

15.3 In Vitro DNA Studies – Sister Chromatid Exchange

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: SCE
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): DNA assay
System of testing [bacterial, non bacterial]: CHO cells
GLP (Y/N): Yes
Year (study performed): 1981

Species/Strain or cell type and or cell line, bacterial or non-bacterial: Chinese hamster ovary cell line

Metabolic activation: S-9 from male rat liver induced by Aroclor 1254.

- Species and cell type: Chinese hamster, ovary cells
- Quantity: 25
- Induced or not induced

Concentrations tested: 0.625 to 20 µl/ml

Statistical Methods: t-test

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations. Detail differences from the guideline followed including the following as appropriate:

- Test Design
  - · Number of replicates: 2
  - · Frequency of Dosing: Once for 2 hours
  - · Positive and negative control groups and treatment: Negative control, solvent control, positive controls: triethylenemelamine without activation; cyclophosphamide with activation
  - · Number of metaphases analyzed: 50 cells
- Solvent: Ethanol
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Student's t-test with dose-response; doubling over negative/solvent control.

RESULTS

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)

- With metabolic activation: Significant increase at 5, 10, 20 µl/ml without dose-response; no doubling – author concluded it was negative
- Without metabolic activation: significant increase with dose-response at 5, 10 and 20 µl/ml; no doubling – author concluded it was positive

Statistical results, as appropriate

Remarks field for Results. Note test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they effect the selection of test concentrations or interpretation of the results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Authors concluded that t-butanol induced chromosomal aberrations *in vitro* in the presence of metabolic activation, but not in its absence.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability

REFERENCES (Free Text): EG&G Mason Research Institute, 1981. An In Vitro Evaluation of t-Butyl Alcohol-ARCONOL, Batch # A209411 to Produce Sister Chromatid Exchanges in Chinese Hamster Ovary Cells. EG&G Mason Final Report 052-399-636-16, June 5, 1981, amended January 30, 1985.

OTHER

Last changed (administrative field for updating): 3/11/02 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

16) REPEATED DOSE TOXICITY

16.1 REPEATED DOSE TOXICITY – CHRONIC STUDY IN RATS

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: NTP standard study
Test type: Chronic study
GLP (Y/N): Yes
Year (study performed): 1986-1988
Species: Rat
Strain: F344/N

Route of administration, oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Duration of test: 103 weeks
Doses/concentration levels: 0, 1.25, 2.5, and 5 mg/ml in males and 0, 2.5, 5, and 10 mg/ml in females
Sex: Both
Exposure period: Continuous 103 weeks
Frequency of treatment: Continuous
Control group and treatment: Yes, same as treated except no TBA in drinking water
Post exposure observation period: No
Statistical methods: Standard

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Test Subjects
  · Age at study initiation: 7 weeks
  · No. of animals per sex per dose - Study Design: 60 males and 60 females

  · Vehicle: Deionized water
  · Satellite groups and reasons they were added: None
  · Clinical observations performed and frequency (clinical pathology, functional observations, etc.): Twice daily, physical examination weekly, blood samples at 15 months for hematology, urinalysis month 15.
  · Organs examined at necropsy (macroscopic and microscopic): Complete set of tissues evaluated for all animals.

RESULTS

NOAEL (NOEL): Not established
LOAEL (LOEL): 1.25 mg/ml in males; 2.5 mg/ml in females
Actual dose received by dose level by sex, if known: 0, 90, 200, and 420 mg/kg in males; 0, 180, 330, and 650 mg/ml in females.
Toxic response/effects by dose level:
At 10 mg/ml – females – decreased survival, reduced body weight, nephropathy and mineralization, renal tubule hyperplasia, transitional epithelium hyperplasia in bladder, increased kidney weights
At 5 mg/ml - males – decreased survival, reduced body weight, mineralization in kidney, transitional epithelium hyperplasia in bladder, increased renal tubule adenoma
females – increased kidney weights, nephropathy
At 2.5 mg/ml – males – decreased survival, mineralization in kidney, transitional epithelium hyperplasia
females – increased kidney weights, nephropathy
At 1.25 mg/ml – males – reduced body weight after week 65.
Statistical results, as appropriate

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

- Body weight (week 101): 454, 387, 374, 344, for males at 0, 1.25, 2.5, and 5 mg/ml
  331, 324, 316, 261, for females at 0, 2.5, 5, and 10 mg/ml
- Food/water consumption:
- Description, severity, time of onset and duration of clinical signs:
- Ophthalmologic findings incidence and severity
- Hematological findings incidence and severity: No treatment-related effects
- Clinical biochemistry findings incidence and severity:
- Mortality and time to death : Survival at 101 week: 12, 10, 4, 2 in males
  29, 28, 26, 17 in females
- Gross pathology incidence and severity: No treatment-related effects
- Organ weight changes: See above
- Histopathology incidence and severity: Kidney tumors (adenomas/carcinomas combined, based on combined standard and step sections: 8, 13, 19*, 13 for 0. 1.25, 2.5, and 5 mg/ml in males. None in females.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Author concluded there was “some evidence of carcinogenicity in males and no evidence of carcinogenicity in females”. Submitter notes that Dr. Gordon Hard reviewed this study and concluded that t-butanol exacerbated chronic progressive nephropathy in male rats and also meet the criteria for alpha -2u-globulin mechanism and that these tumors are not relevant for human risk assessment. (Hard, G. 2001. Expert Evaluation of Renal Effects of tert-Butyl Alcohol in Rats and Mice. Report to Lyondell Chemical Company, Houston Texas, pp. 1-12.)

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability

REFERENCES (Free Text): National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

Cirvello, J.D., Radovsky, A., Heath, J.E., Farnell, D.R., and Lindamood, III, C., 1995. Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. Toxicol. Ind. Health 11: 151-165.

OTHER

Last changed (administrative field for updating): 3/1202 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks [Note - Use for any other comments necessary for clarification.]

16.2 REPEATED DOSE TOXICITY – CHRONIC STUDY IN MICE

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: NTP standard study
Test type: 2-year
GLP (Y/N): Yes
Year (study performed): 1986-1988
Species: Mouse
Strain: B6C3F1
Route of administration, oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Duration of test: 103 weeks
Doses/concentration levels: 0, 5, 10, and 20 mg/ml
Sex: Both
Exposure period: Continuous 103 weeks
Frequency of treatment: Continuous
Control group and treatment: Yes, same as treated except no TBA in drinking water
Post exposure observation period: No
Statistical methods: Standard

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Test Subjects
  · Age at study initiation: 7 weeks
  · No. of animals per sex per dose - Study Design: 60 males and 60 females

  · Vehicle: Deionized water

· Satellite groups and reasons they were added: None
· Clinical observations performed and frequency (clinical pathology, functional observations, etc.): Twice daily, physical examination weekly,
· Organs examined at necropsy (macroscopic and microscopic): Complete set of tissues evaluated for mice.

RESULTS

NOAEL (NOEL): 5 mg/ml (~525 mg/kg/day)
LOAEL (LOEL): 10 mg/ml (~1030 mg/kg/day)
Actual dose received by dose level by sex, if known: 0, 540, 1040, and 2070 mg/kg in males; 0, 510, 1020, and 2110 mg/kg in females.
Toxic response/effects by dose level:
At 20 mg/ml – males – reduced survival, reduced body weight, increased thyroid follicular cell hyperplasia
females – reduced body weight, increased follicular cell hyperplasia, increased thyroid follicular cell adenoma
At 10 mg/ml – males – increased follicular cell hyperplasia, marginally increased follicular cell adenoma
females – increased follicular cell hyperplasia
At 5 mg/ml - males – No effect
females – No effect
Statistical results, as appropriate

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

- Body weight: 53, 53, 51, 50*, for males at 0, 5, 10, and 20 mg/ml at week 81, difference diminished after this
60, 58, 57, 52*, for females at 0, 5, 10, and 20 mg/ml at week 81
*statistically significant

- Food/water consumption: No effect
- Description, severity, time of onset and duration of clinical signs: None
- Ophthalmologic findings incidence and severity: Not examined
- Hematological findings incidence and severity: Not examined
- Clinical biochemistry findings incidence and severity: Not examined
- Mortality and time to death: Survival at 101 week: 34, 44, 41, 23 in males
43, 43, 46, 46 in females
- Gross pathology incidence and severity: No treatment-related effects
- Organ weight changes: See above
- Histopathology incidence and severity:
Increased chronic inflammation of urinary bladder at 20 mg/ml in males and females
Increased follicular cell adenoma in females at 20 mg/ml (2/58, 3/60, 2/59, 9/59)

Marginally increased follicular cell adenoma in males (1/60, 0/59, 4/59, 2/57);1 carcinoma seen at 20 mg/ml
Increased follicular cell hyperplasia males and females at 10 and 20 mg/ml

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Author concluded there was equivocal evidence of carcinogenicity in males and some evidence of carcinogenicity in females.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

Remarks field for Data Reliability

REFERENCES (Free Text): National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

Cirvello, J.D., Radovsky, A., Heath, J.E., Farnell, D.R., and Lindamood, III, C., 1995. Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. Toxicol. Ind. Health 11: 151-165.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks [Note - Use for any other comments necessary for clarification.]

16.3 REPEATED DOSE TOXICITY – SUBCHRONIC STUDY IN RATS

TEST SUBSTANCE

Identity: t-Butyl alcohol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: NTP standard study

Test type: 13 weeks
GLP (Y/N): Yes
Year (study performed): 1985
Species: Rat
Strain: F344/N
Route of administration, oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Duration of test: 92-94 days
Doses/concentration levels: 0, 2.5, 5, 10, 20 and 40 mg/ml
Sex: Both
Exposure period: Continuous 92-94 days
Frequency of treatment: Continuous
Control group and treatment: Yes, same as treated except no TBA in drinking water
Post exposure observation period: No
Statistical methods: Standard

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Test Subjects
  - · Age at study initiation: 6 weeks
  - · No. of animals per sex per dose - Study Design: 10 males and 10 females
  - · Vehicle: Deionized water
  - · Satellite groups and reasons they were added: None
  - · Clinical observations performed and frequency (clinical pathology, functional observations, etc.): Twice daily, physical examination weekly, blood samples at 2 and 13 weeks for hematology and clinical chemistry, urinalysis weeks 2 and 13.
  - · Organs examined at necropsy (macroscopic and microscopic): Complete set of tissues evaluated for rats in 0, 20, and 40 mg/ml dose groups; target organs in lower dose groups.

RESULTS

NOAEL (NOEL): 2.5 mg/ml in males, except for kidney for which a NOAEL was not established; 5 mg/ml in females
LOAEL (LOEL): 5 mg/ml in males; 10 mg/ml in females
Actual dose received by dose level by sex, if known: 0, 230, 490, 840, 1520, and 3610 mg/kg in males; 0, 290, 590, 850, 1560, and 3620 mg/ml in females.
Toxic response/effects by dose level:
At 40 mg/ml – death of all males and 6 of 10 females, nephropathy, transitional epithelial hyperplasia in urinary bladder, increased liver and kidney weights
At 20 mg/ml – males – reduced body weight, nephropathy, hyaline droplet accumulation, increased liver and kidney weights
females – nephropathy, increased liver and kidney weights

At 10 mg/ml – males – reduced body weight, nephropathy, hyaline droplet accumulation, increased liver and kidney weights
females – nephropathy, increased liver and kidney weights
At 5 mg/ml - males – reduced body weight, nephropathy, hyaline droplet accumulation, increased kidney weights
females –increased liver and kidney weights
At 2.5 mg/ml – males - nephropathy, hyaline droplet accumulation, increased kidney weights
Females –increased liver and kidney weights

Statistical results, as appropriate

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

- Body weight: 355, 341, 339, 313, 294 g for males at 0, 2.5, 5, 10, and 20 mg/ml
179, 183, 180, 181, 176, 141 g for females at 0, 2.5, 5, 10, 20, and 40 mg/ml
- Food/water consumption:
- Description, severity, time of onset and duration of clinical signs: No effects
- Ophthalmologic findings incidence and severity: Not examined
- Hematological findings incidence and severity: Decreased RBC count and hemoglobin in males at 10 and 20 mg/ml; no differences in females
- Clinical biochemistry findings incidence and severity: Increased sorbitol dehydrogenase in males at 10 and 20 mg/ml; increase alanine aminotransferase in females at 20 and 40 mg/ml.
- Mortality and time to death: 10 /10 males at 40 mg/ml weeks 4, 5, 5, 6, 7, 8, 8, 8, 12, 12
6 / 10 females at 40 mg/ml weeks 2, 8, 8 10 , 12, 12
- Gross pathology incidence and severity: Calculi in urinary bladder in males at 40 mg/ml
- Organ weight changes: See above
- Histopathology incidence and severity: See above

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Author concluded 5 mg/ml in males and 10 mg/ml in females were maximum tolerated doses for 2 year study. Submitter notes That Dr. Michael McClain reviewed the thyroid effects in mice. He concluded that the increased thyroid tumors are compatible with a proliferative response seconday to hormone imbalance caused by microsomal enzyme induction. (McClain, R.M. 2001. Assessment of the Thyroid Follicular Cell Tumor Findings from Toxicity and Carcinogenicity Studies with tert-Butyl Alcohol in B6C3F1 Mice, Report to Lyondell Chemical Company, Houston, Texas, pp. 1-14.)

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1

Remarks field for Data Reliability: Not regarded as key study because chronic study available.

REFERENCES (Free Text): National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

Lindamood *et al.*, 1992. Subchronic Toxicity Studies of t-butyl alcohol in Rats and Mice. Fund. Appl. Toxicol. 19: 91-100.

OTHER

Last changed (administrative field for updating): 3/12/02 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks [Note - Use for any other comments necessary for clarification.]

16.4 REPEATED DOSE TOXICITY – SUBCHRONIC STUDY IN MICE

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: NTP standard study
Test type: 13 weeks
GLP (Y/N): Yes
Year (study performed): 1985
Species: Mouse
Strain: B6C3F1
Route of administration, oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Duration of test: 94-95 days
Doses/concentration levels: 0, 2.5, 5, 10, 20 and 40 mg/ml
Sex: Both
Exposure period: Continuous 94-95 days
Frequency of treatment: Continuous
Control group and treatment: Yes, same as treated except no TBA in drinking water

Post exposure observation period: No
Statistical methods: Standard

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Test Subjects
  - · Age at study initiation: 6 weeks
  - · No. of animals per sex per dose - Study Design: 10 males and 10 females

  - · Vehicle: Deionized water
  - · Satellite groups and reasons they were added: None
  - · Clinical observations performed and frequency (clinical pathology, functional observations, etc.): Twice daily, physical examination weekly, blood samples at 2 and 13 weeks for hematology.
  - · Organs examined at necropsy (macroscopic and microscopic): Complete set of tissues evaluated for mice in 0, 20, and 40 mg/ml dose groups; target organs in lower dose groups.

RESULTS

NOAEL (NOEL): 10 mg/ml in males; 10 mg/ml in females
LOAEL (LOEL): 20 mg/ml in males; 20 mg/ml in females
Actual dose received by dose level by sex, if known: 0, 350, 640, 1590, 3940, and 8210 mg/kg in males; 0, 500, 820, 1660, 6430, and 11,620 mg/kg in females.
Toxic response/effects by dose level:
At 40 mg/ml – males - death of 6 of 10, reduced body weight, transitional epithelial hyperplasia in urinary bladder
females – death of 4 of 10, reduced body weight, increased liver and kidney weights, transitional epithelial hyperplasia in urinary bladder
At 20 mg/ml – males – reduced body weight, transitional epithelial hyperplasia in urinary bladder
females – death of 1 of 10
At 10 mg/ml – males - No effect
females – No effect
At 5 mg/ml - males – No effect
females – No effect
At 2.5 mg/ml – males - No effect
females – No effect
Statistical results, as appropriate

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen.

- Body weight: 38, 38, 38, 37, 33, and 29 g for males at 0, 2.5, 5, 10, 20, and 40 mg/ml
  30, 31, 29, 31, 28, and 25 g for females at 0, 2.5, 5, 10, 20, and 40 mg/ml
- Food/water consumption:
- Description, severity, time of onset and duration of clinical signs: No treatment-related effects other than related to deaths
- Ophthalmologic findings incidence and severity: Not examined
- Hematological findings incidence and severity: Not examined
- Clinical biochemistry findings incidence and severity: Not examined
- Mortality and time to death: 6 of 10 males at 40 mg/ml weeks 4, 5, 7, 7, 9, 13
  4 of 10 females at 40 mg/ml weeks 2, 5, 9, 9
  1 of 10 females at 20 mg/ml week 10
- Gross pathology incidence and severity: Increased thickness of urinary bladder in males at 20 and 40 mg/ml
- Organ weight changes: Increased liver and kidney weights in females at 40 mg/ml
- Histopathology incidence and severity: Hyperplasia of the urinary bladder transitional epithelium

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Author concluded 20 mg/ml in males and females were maximum tolerated doses for 2 year study.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1

Remarks field for Data Reliability: Not regarded as key study because chronic study is available.

REFERENCES (Free Text): National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

Lindamood *et al.* , 1992. Subchronic Toxicity Studies of t-butyl alcohol in Rats and Mice. Fund. Appl. Toxicol. 19: 91-100.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks [Note - Use for any other comments necessary for clarification.]

17) TOXICITY TO REPRODUCTION

17) TOXICITY TO REPRODUCTION

17. 1 REPRODUCTION/DEVELOPMENTAL TOXICITY SCREEN

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.) Commercial t-butanol, 99.6% pure.

METHOD

Method/guideline followed: OECD 421, enhanced with longer premating exposure and sperm evaluation, 1 male and 1 female F1 pup from each litter exposed post natal days 21-27.
Type (one generation, two generation, etc.): One-generation
GLP (Y/N): Yes
Year (study performed): 2003
Species: Rat
Strain: CRL CD (Sprague-Dawley derived)
Route of administration - oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral (gavage)
Doses/concentration levels: 0, 64, 160, 400, and 1000 mg/kg/day t-butanol in distilled/deionized water administered at 5ml/kg
Sex: Males and Females
Exposure period: Four weeks pre-mating; males total of 9 weeks; females through gestation and lactation. F1 pups (1male, 1 female/litter) exposed 1 week.
Frequency of treatment: Once daily
Control group and treatment: Negative control: distilled/deionized water at 5 ml/kg.
Duration of test: Males 9 weeks; Females through postnatal day 21: 10-11 weeks overall.
Statistical methods: Bartlett's for equalency of variances; ANOVA, Dunnett's; Fisher Exact Test with Bonferroni correction for incidence data.

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age at study initiation: 8 weeks old
- Number of animals per dose per sex: 12 males and 12 females

- Vehicle: Distilled/deionized water
- Clinical observations performed and frequency: Daily, 4 hours after dosing
- Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): 1 male/1 female each night in male cage until mated; sperm plug or sperm in vaginal smear as evidence of mating.
- Parameters assessed during study (maternal and fetal): Paternal: clinical signs, body weight, feed consumption, sperm evaluation. Maternal: clinical signs, body weight, feed consumption, length of gestation. Fetal: survival, body weight.
- Organs examined at necropsy (macroscopic and microscopic): Macroscopic: all organs and body cavities; organ weights: liver, kidney, males: testes, epididymides; microscopic: testis, epididymis, ovary, thyroid.

RESULTS

NOAEL (NOEL) and LOAEL (LOEL) paternal and maternal toxicity: 160 mg/kg/day
NOAEL (NOEL) and LOAEL (LOEL) reproductive/developmental toxicity:
400 g/kg/day
Actual dose received by dose level by sex if known: targeted doses – by gavage
Statistical results, as appropriate

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following when there are dose related effects if available:

- Body weight: In males at 1000 mg/kg/day, there was an initial reduction in body weight gain, which remained as a 5-7% deficit in weight until termination. During late gestation, there was a reduction in weight gain in females.
- Food/water consumption: No effect
- Description, severity, time of onset and duration of clinical signs: At 1000 mg/kg/day, there was transient lethargy, and ataxia. At 400 mg/kg/day similar effects were seen in a few females during weeks 2-4.
- Organ Weights: At all dose levels there was a dose-related increase in male kidney weight (8, 13, 15, and 30%, at 64, 160, 400 and 1000 mg/kg/day, respectively). At 1000 mg/kg/day, males also had increased liver weights (15%).
- Gross and Microscopic Pathology: There were no treatment-related gross or microscopic pathology findings.
- Fertility: There was no effect on mating or fertility; 11-12 females in each group became pregnant and all delivered a live litter.
- Estrus cycles and gestation length:
  All but three females mated at the first estrus (one each in 0, 64, and 160 mg/kg/day groups)
  There was a questionable increase in gestation length at 400 and 1000 mg/kg/day. All females delivered within the normal range of 21-23 days; however, 6 of 11 females at 1000 mg/kg/day and 5 of 12 at 400 mg/kg/day vs. no more than 20% in any of the control and lowest two treatment groups delivered on day 23.
- Sperm: No effect on sperm motility or sperm morphology.

- F1 Litter Size and Survival: There was no effect on the number of implantation sites per pregnancy. At 1000 mg/kg/day, there was a significant reduction in the number of live born pups and an increase in the number of still born pups. The mean litter sizes on postnatal day 1 were: 15.2, 13.8, 13.5, 14.1 and 10.2** for 0, 64, 160, 400 and 1000 mg/kg/day, respectively; **= p<0.01. Following reduction of litter sizes to 10 pups/litter on postnatal day 4, postnatal day 21 mean litter sizes were: 10.0, 9.9, 9.3, 9.9, and 7.6**.

-F1 Body Weight: F1 offspring born to dams treated at 1000 mg/kg/day had lower body weight on day 1, which continued throughout gestation. At weaning, males weighed 11% less than control and females 6% less than control.

-F1 Directly Exposed Pups: There were no clinical signs of toxicity at any dose during the one week of treatment. The decreased body weight at weaning was maintained without change during the week.

CONCLUSIONS: The NOAEL for paternal and maternal toxicity was 160 mg/kg/day t-butanol. Maternally toxic doses of t-butanol (1000 mg/kg/day) resulted in decreased survival and body weight of pups. The NOAEL for reproduction/development was 400 mg/kg/day. Direct exposure of pups for one week after weaning did not exhibit further toxicity.

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Conclusions of the author.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1

Remarks field for Data Reliability: OECD guideline screening study.

REFERENCES (Free Text): Hazelden, K.A., (2004). TBA: Reproduction/Developmental Toxicity Screening in Rats; Study Number 03-4254. Report from Huntingdon Life Sciences, Princeton, New Jersey.

OTHER

Last changed (administrative field for updating): 4/23/04 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks [Note - Use for any other comments necessary for clarification.]

17. 2 TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.)

METHOD

Method/guideline followed: None
Type (one generation, two generation, etc.): Evaluation of sex organs in subchronic study
GLP (Y/N): Yes
Year (study performed): 1986
Species: Rat and Mouse
Strain: F344/N and B6C3F1
Route of administration - oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Doses/concentration levels: 0, 2.5, 5, 10, 20 and 40 mg/ml
Sex: Both
Control group and treatment: Yes, same as treated except no TBA in drinking water
Frequency of treatment: Continuous
Duration of test: 92-96 days
Statistical methods

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

Sperm morphology, motility, concentration
Estrus cycle length, percent in stages
Organs examined at necropsy (macroscopic and microscopic): Testis, epididymis, ovary, uterus

RESULTS

NOAEL (NOEL) and LOAEL (LOEL): NOAEL for effects on reproductive organs/function =
Rats: males -3610 mg/kg/day; females – 3620 mg/kg/day;
Mice: males – 8210 mg/kg/day; females – 6430 mg/kg/day.
LOAEL female mice only 11,620 mg/kg/day
Actual dose received by dose level by sex if known: rats: 0, 230, 490, 840, 1520, and 3610 mg/kg in males; 0, 290, 590, 850, 1560, and 3620 mg/ml in females; mice: 0, 350, 640, 1590, 3940, and 8210 mg/kg in males; 0, 500, 820, 1660, 6430, and 11,620 mg/ml in females.
Statistical results, as appropriate

Remarks field for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following when there are dose related effects if available:

- Body weight: Rats: decreased in males at 5, 10, 20 mg/ml; females at 40 mg/ml; Mice: decreased in males at 20 and 40 mg/ml and in females at 40 mg/ml.
- Food/water consumption:
- Description, severity, time of onset and duration of clinical signs:
- Changes in estrus cycles:

Rats: No effect on estrous cycle length or percentage of time spent in the various estrous stages.

Mice: No effect on percentage of time spent in various stages of estrous, but increased estrous cycle length at 40 mg/ml (40% died, also reduced body weight).

- Effects on sperm:

Rats and mice: No effect on sperm motility or sperm morphology.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. The significance of the increased estrus cycle length observed in female mice receiving 11,620 mg/kg (40 mg/ml in water) t-butanol with a 40% rate of mortality is questionable. Altered estrus cycle length (typically increased) is common in female animals with altered homeostasis due to systemic toxicity. Of significance was the lack of altered estrus cycle length in female mice receiving 6430 mg/kg (20 mg/ml in water), with a 10% incidence of mortality.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2

Remarks field for Data Reliability: studies well done, but assess endpoints of reproductive organs, not reproduction

REFERENCES (Free Text): National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

18) DEVELOPMENTAL TOXICITY/TERATOGENICITY

18.1 DEVELOPMENTAL TOXICITY STUDY IN RATS

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.): Greater than 99% pure by gas chromatography

METHOD

Method/guideline followed: Teratogenicity study
GLP (Y/N): No data
Year (study performed): Not stated, submitted for publication March, 1988.
Species: Rat
Strain: Charles River Sprague-Dawley derived
Route of administration - oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: inhalation, vapor
Doses/concentration levels: 0, 2000, 3500, and 5000 ppm
Sex: Females
Exposure period: Days 1-19 of gestation
Frequency of treatment: Daily, 7 hours/day
Control group and treatment: Clean air
Duration of test: Through gestation day 19
Statistical methods: multivariate analysis, Kruskal-Wallis test, t-test with Bonferroni correction when appropriate

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age at study initiation: age not provided; weight 200-300 grams at mating
- Number of animals per dose per sex: 15-20 mated females
- Vehicle: Clean air
- Clinical observations performed and frequency: Not stated
- Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): 1 male/1 females until mated; sperm plug or vaginal sperm
- Parameters assessed during study (maternal and fetal): Maternal: body weight, food consumption. Fetal: resorptions, external, skeletal, visceral.
- Organs examined at necropsy (macroscopic and microscopic): skeletal, visceral

RESULTS

NOAEL (NOEL) and LOAEL (LOEL) maternal toxicity: No NOAEL.
LOAEL = 2000 ppm – decreased locomotor activity
NOAEL (NOEL) and LOAEL (LOEL) developmental toxicity
Actual dose received by dose level by sex if available

Maternal data with dose level (with NOAEL value). Provide at a minimum qualitative descriptions of responses where dose related effects were seen.:

Decreased body weight gain at 5000 ppm (56 g vs. 120 g); NOAEL = 3500 ppm (95 g).
Decreased feed consumption at 5000 ppm (278 g vs. 361 g, Day 1 – 20); NOAEL = 3500 ppm (354 g).
Unsteady Gait at end of exposure at 3500 and 5000 ppm; impaired locomotor activity at 2000 ppm.

Fetal data with dose level (with NOAEL value). Provide at a minimum qualitative descriptions of responses where dose related effects were seen:

Reduced fetal weights at all exposure concentrations:
Female: 3.2, 2.9*, 2.8*, 2.2* g/fetus at 0, 2000, 3500, and 5000 ppm
Male: 3.4, 3.1*, 3.0*, 2.3* g/fetus at 0, 2000, 3500, and 5000 ppm
Increased fetuses (but not litters) with skeletal variations (largely delayed ossification) at 3500 and 5000.
Variations in 10/15, 14/17, 14/14 and 12/12 litters and in 18, 35, 53*, and 76* fetuses at 0, 2000, 3500, 5000 ppm.

*p<0.05

Statistical results, as appropriate:

At 5000 ppm: Decreased maternal weight gain, weeks 1, 2, 3; decreased maternal feed consumption weeks 1 and 2; decreased fetal weight, males and females; increased fetuses with skeletal variations.
At 3500 ppm: Decreased fetal weight; increased fetuses with skeletal variations.
At 2000 ppm: Decreased fetal weight.

Remarks for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following when there are dose related effects if available: Maternal data, provide at a minimum qualitative descriptions of responses where dose related effects were seen.

- Mortality and day of death: None
- Number pregnant per dose level: 15, 18, 15, 13 at 0, 2000, 3500, 5000 ppm
- Number aborting: None
- Number of resorptions, early/late if available:
  Early+late: 1.2±1.2, 1.2±1.1, 0.9±1.0, 1.1±0.9 resorptions/litter at 0, 2000, 3500, 5000 ppm.
- Number of implantations: Not stated
- Pre and post implantation loss, if available: Not stated
- Number of corpora lutea (recommended): 16±2, 16±2, 16±2, 16±2 at 0, 2000, 3500, and 5000 ppm.
- Duration of Pregnancy: Not applicable; terminated on gestational day 20
- Body weight: Not given: Body weight gain to gestation day 20: 120, 104, 95, 56 g (interpolated from graph) at 0, 2000, 3500, 5000 ppm
- Food/water consumption: Feed Consumption Days 1-20: 361, 354, 350, 278 g/animal at 0, 2000, 3500, 5000 ppm.
- Description, severity, time of onset and duration of clinical signs: Not reported

- Fetal data, provide at a minimum qualitative descriptions of responses where dose related effects were seen:
· Litter size and weights

	0	2000	3500	5000 ppm
Live fetuses/litter =	13±2	13±4	15±2	14±2
Male fetus wt (g) =	3.4±0.21	3.1±0.19*	3.0±0.248	2.3±0.34*
Female fetus wt(g)=	3.2±0.23	2.9±0.20*	2.8±0.20*	2.2±0.34*

· Sex ratio: Percent females/litter: 56±16, 53±13, 50±12, 46±16 at 0, 2000, 3500, 5000 ppm
· Grossly visible abnormalities, external, soft tissue and skeletal abnormalities:
External examinations: No external malformations in any group
Skeletal malformations in 0/15,0/17,2/14,4/12 litters at 0, 2000, 3500, 5000 ppm
Skeletal variations in 10/15,14/17,14/14,12/12 litters at 0, 2000, 3500, 5000 ppm
Visceral malformations in 1/15,1/17,2/14,1/12 litters at 0, 2000, 3500, 5000 ppm
Visceral variations in 6/15,4/17,6/14,12/12 litters at 0, 2000, 3500, 5000 ppm

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. The submitter concludes there were developmental delays in fetuses whose dams experienced significant maternal toxicity from exposure to 2000 to 5000 ppm t-butanol during gestation days 1-19. There were no malformations.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1

Remarks field for Data Reliability: Study appears to meet current guidelines, although details of experimental procedure and results are slightly limited.

REFERENCES (Free Text): Nelson, B.K., Brightwell, W.S., Khan, A., Burg, J.R., Goad, P.T., (1989). Lack of selective developmental toxicity of three butanol isomers administered by inhalation to rats. Fundam. Appl. Toxicol. 12: 469-479.

OTHER

Last changed (administrative field for updating): 4/12/04by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.) Summarized, along with 12 other alcohols in Nelson, B.K., Brightwell, W.S., Krieg, Jr., E.F. (1996). Developmental toxicology of industrial alcohols: A summary of 13 alcohols administered by inhalation to rats. Intl. J. Occup. Med. Immunol. Toxicol. 5: 29-42. (first published in Toxicol. Ind. Health 6: 373-387 (1990).

18.2 POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN MICE

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.): No description of source or purity

METHOD

Method/guideline followed: Teratogenicity study with developmental landmarks
GLP (Y/N): No data
Year (study performed): Not stated, accepted for publication 1981
Species: Mice
Strain: Swiss Webster
Route of administration - oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral (in liquid diet)
Doses/concentration levels: 0.5, 0.75 and 1.0 % in diet.
Sex: Females
Exposure period: Days 6-20 of gestation
Frequency of treatment: Continuous
Control group and treatment: Negative control: Liquid diet without t-butanol; Positive control: 3.6% ethanol
Duration of test: Through postnatal day 22
Statistical methods: SAS package: t-test, linear regression

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age at study initiation: 8 to 10 weeks old
- Number of animals per dose per sex: 15
- Vehicle: Modified Lieber and Decarli liquid diet
- Clinical observations performed and frequency: Not stated
- Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): 1 male/3 females until mated; sperm plug
- Parameters assessed during study (maternal and fetal): Maternal: body weight, length of gestation
- Organs examined at necropsy (macroscopic and microscopic): Not stated

RESULTS

NOAEL (NOEL) and LOAEL (LOEL) maternal toxicity: Not stated
NOAEL (NOEL) and LOAEL (LOEL) developmental toxicity
Actual dose received by dose level by sex if available
Maternal data with dose level (with NOAEL value). Provide at a minimum qualitative descriptions of responses where dose related effects were seen.:

Authors report “significant postnatal maternal nutritional and behavioral factors affecting lactation and/or nesting behavior were evident at the higher concentrations of alcohol” without description or quantization of those effects. Decreased body weight at 0.75 and 1.0 % t-butanol (TBA).

Fetal data with dose level (with NOAEL value). Provide at a minimum qualitative descriptions of responses where dose related effects were seen:

Two-day delay in eye opening at 1% TBA, reduced pup weight at 1 % TBA, less obvious in pups that were cross fostered; effects were reported for cliff avoidance, righting reflex, open field activity, and roto-rod performance in pups from TBA exposed dams, but the results were not compared to control values, so a NOAEL was not reported.

Statistical results, as appropriate

Remarks for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following when there are dose related effects if available: Maternal data, provide at a minimum qualitative descriptions of responses where dose related effects were seen.

- Mortality and day of death: None
- Number pregnant per dose level: Not stated
- Number aborting: Not stated
- Number of resorptions, early/late if available: Not stated
- Number of implantations: Not stated
- Pre and post implantation loss, if available: Not stated
- Number of corpora lutea (recommended): Not stated
- Duration of Pregnancy: Not applicable; terminated on gestational day 20
- Body weight: At gestational day 20: Control - 43.3 g, 0.5% TBA – 44.3 g; 0.75% TBA – 41.0 g; 1.0% TBA – 39.0 g
- Food/water consumption: Pair-fed from 1% TBA consumption
- Description, severity, time of onset and duration of clinical signs: Not reported
- Fetal data, provide at a minimum qualitative descriptions of responses where dose related effects were seen:

	0	0.5%	0.75%	1.0%
· Litter size and weights: Neonates/litter =	10.4±4.0	10.3±4.4	7.4±2.3	5.3±2.8
Fetal wt, day2 =	1.78±0.21	1.66±0.24	1.45±0.14	1.10±0.10

· Number viable (number alive and number dead): Total Number Stillborn: 3, 6, 14, 20 at 0, 0.5, 0.75 and 1.0% TBA
· Sex ratio: Not reported
· Postnatal growth (depending on protocol): at postnatal day 10 (estimated from graph): 6.9, 6.5, 6.0 and 4.0 g/pup for 0, 0.5, 0.75, and 1.0% TBA
· Postnatal survival (depending on protocol): Not reported
· Grossly visible abnormalities, external, soft tissue and skeletal abnormalities: Not reported

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. The submitter concludes there were developmental delays in pups whose dams experienced significant maternal toxicity from exposure to 0.75 or 1.0% t-butanol in the liquid diet.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2

Remarks field for Data Reliability: Not guideline study, incomplete evaluation; not able to calculate actual dose (mg/kg) of t-butanol.

REFERENCES (Free Text): Daniel, M.A. and Evans, M.A., 1982. Quantitative Comparison of Maternal Ethanol and Maternal Tertiary Butanol Diet on Postnatal Development. J. Pharm. Exp. Therap. 222: 294-300.

OTHER

Last changed (administrative field for updating): 11/21/01by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)

18.3 DEVELOPMENTAL TOXICITY/TERATOGENICITY in MICE

TEST SUBSTANCE

Identity: t-Butanol

Remarks field for Test Substance (Use for any pertinent, test substance-specific remarks.) No description of test material in paper

METHOD

Method/guideline followed : Not specified
GLP (Y/N): No data
Year (study performed): Not specified
Species: Mouse
Strain: CBA/J and C57BL/6J
Route of administration - oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other : Gavage
Doses/concentration levels: 10.5 mmole/kg twice per day (1550 mg/kg/day)
Sex: Female
Exposure period : Days 6-18 of gestation
Frequency of treatment: Twice daily

Control group and treatment: Similar size group for each strain; received tap water by gavage twice daily
Duration of test: Through day 18 of gestation
Statistical methods: t-test, chi squared

Remarks field for Test Conditions. Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate:

- Age at study initiation: 25-30 weeks old
- Number of animals per dose per sex: CBA/J: control = 7 dams; TBA = 12 dams; C57BL/6J: control = 5 dams; TBA = 9 dams
- Vehicle: Tap water
- Clinical observations performed and frequency: Not stated
- Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): Not stated
- Parameters assessed during study (maternal and fetal): Not stated
- Organs examined at necropsy (macroscopic and microscopic): Uterus, fetuses

RESULTS

NOAEL (NOEL) and LOAEL (LOEL) maternal toxicity: Not reported
NOAEL (NOEL) and LOAEL (LOEL) developmental toxicity: NOAEL not established
Actual dose received by dose level by sex if available: 1550 mg/kg/day

Remarks for Results. Describe additional information that may be needed to adequately assess data for reliability and use include the following when there are dose related effects if available: Maternal data, provide at a minimum qualitative descriptions of responses where dose related effects were seen.

- Mortality and day of death: Not stated
- Number pregnant per dose level: CBA/J: 7 and 12; C57BL/6J: 5 and 9
- Number aborting: Not reported
- Number of resorptions, early/late if available: mean/litter: CBA: 1.42±0.72 control; 3.09±0.57 TBA; C57Bl/6J: 0.80±0.57 control; 4.22±1.29 TBA
- Number of implantations: (Mean) – CBA: 9.4; 7.8; C57BL/6 8.8; 7.6
- Pre and post implantation loss, if available: Not reported
- Number of corpora lutea (recommended): Not reported
- Duration of Pregnancy: Terminated day 18
- Body weight: Not reported
- Food/water consumption: Not reported
- Description, severity, time of onset and duration of clinical signs: Not reported
- Organ weight changes, particularly effects on total uterine weight: Not reported
- Histopathology incidence and severity: Not reported
- Fetal data, provide at a minimum qualitative descriptions of responses where dose related effects were seen CBA C57BL/6

	control	TBA	control	TBA
· Litter size: Live fetuses: mean/litter	8.00±1.00	4.75±1.00	8.00±0.45	3.33±1.44
· Litter weights: mean/litter (g±sem)	–0.80±0.01	0.77±0.02	0.94±0.02	0.90±0.03
· Resorptions (mean ±sem):	1.42±0.72	3.09±0.57	0.80±0.49	4.22±1.29

· Sex ratio: Not reported

· Grossly visible abnormalities, external, soft tissue and skeletal abnormalities: No significant differences

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Authors concluded that TBA at 1550 mg/kg/day caused increased resorptions, but not developmental anomalies.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2

Remarks field for Data Reliability: Not guideline study, incomplete evaluation.

REFERENCES (Free Text): Faulkner, T.P., Wiechart, J.D., Hartman, D.M., and Hussain, A.S., 1989. The effects of prenatal tertiary butanol administration in CBA/J and C57BL/6J mice. Life Sci. 45: 1989-1995.

OTHER

Last changed (administrative field for updating): 3/11/02 by ToxWorks
Order number for sorting (administrative field)

Remarks field for General Remarks (Use for any other comments necessary for clarification.)